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Supplemental Information

An Interleukin-21- Interleukin-10-STAT3 Pathway Is Critical for Functional Maturation of Memory CD8$^+$ T Cells

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Figure S1. Kinetics, phenotypes and tissue distribution of *Stat3*$^{+/+}$ or *Stat3*$^{-/-}$ LCMV-specific CD8$^+$ T cells following LCMV infection. (A) Line graphs show the number of total, KLRG1^{lo} IL-7R^{hi} and KLRG1^{hi} IL-7R^{lo} D^{b}NP$_{396-404}$-specific CD8$^+$ T cells in spleen at day 8, 40 and 80 p.i. (B) Dot plots show the expression of KLRG1 and IL-7R in D^{b}NP$_{396-404}$ tetramer$^+$ CD8$^+$ T cells at day 8 and 80 p.i. (C) Histograms show the expression level (MFI) of CD27, CD122, Granzyme B and BCL-2, and percentage of CD62L$^+$, IFNγ^+ and TNFα^+ in LCMV-specific *Stat3*$^{+/+}$ (shaded) and *Stat3*$^{-/-}$ (dashed line) CD8$^+$ T cells at day 8 p.i. (D-E) *Stat3*$^{+/+}$ (solid bars) and *Stat3*$^{-/-}$ (open bars) mice (D) or mice containing *Stat3*$^{+/+}$ or *Stat3*$^{-/-}$ P14 CD8$^+$ T cells (E) were infected with LCMV and the number of D^{b}GP$_{33-41}$-specific CD8$^+$ T cells in spleen (SPL), lymph nodes (LN), bone marrow (BM), liver (LV) and lung (LG) were enumerated at ~day 80. Data shown are representative of three independent experiments.

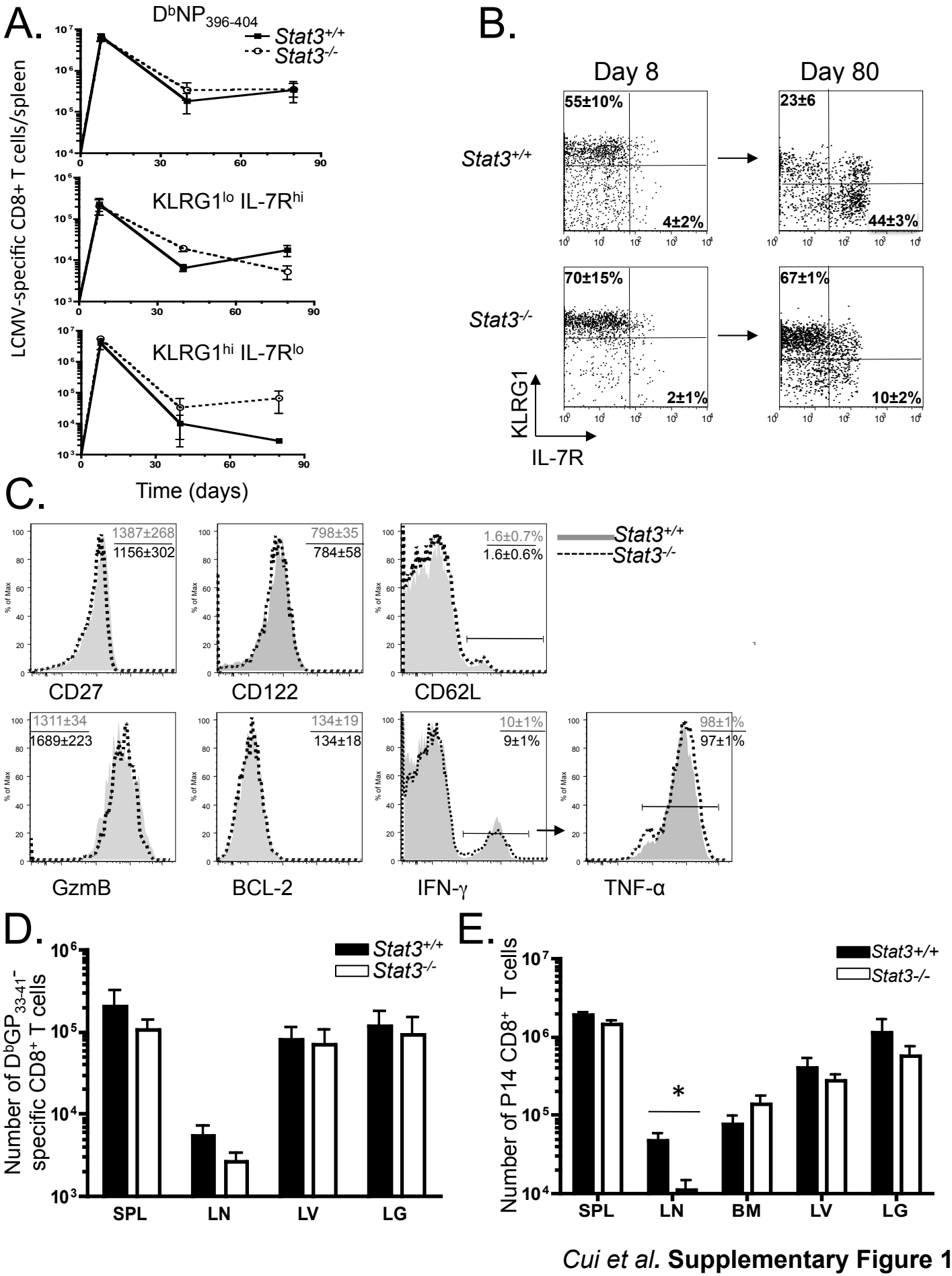
Figure S2. STAT3 is required for P14 memory CD8$^+$ T cell self-renewal and protective immunity. (A) Mice that received small number (5x10^4) of naïve P14 *Stat3*$^{+/+}$ and *Stat3*$^{-/-}$ CD8$^+$ T cells were infected with LCMV and BrdU was given in their drinking water from days 70-80 p.i. BrdU incorporation (±SEM) in P14 *Stat3*$^{+/+}$ (solid line) and *Stat3*$^{-/-}$ (dashed line) memory CD8$^+$ T cell is shown in histograms. (B-C) Equal numbers (5x10^4) of P14 *Stat3*$^{+/+}$ and *Stat3*$^{-/-}$ memory CD8$^+$ T cells were adoptively transferred into naïve hosts that were subsequently infected by 2x10^6 pfu LCMV Clone 13. Bar graphs show the numbers of secondary effector CD8$^+$ T cells (B) and viral titers (C) in the serum at day 7 p.i. Data shown are representative of three independent experiments (* denotes $p<0.05$, ** denotes $p<0.01$).

Figure S3. Blockade of IL-10 and IL-21 does not impair effector CD8$^+$ T cell differentiation. (A) Histogram shows the amount of pSTAT3$_{705}$ in P14 effector CD8$^+$ T cells from day 7 LCMV infected mice after *in vitro* incubation with IL-10 and IL-21 for 30

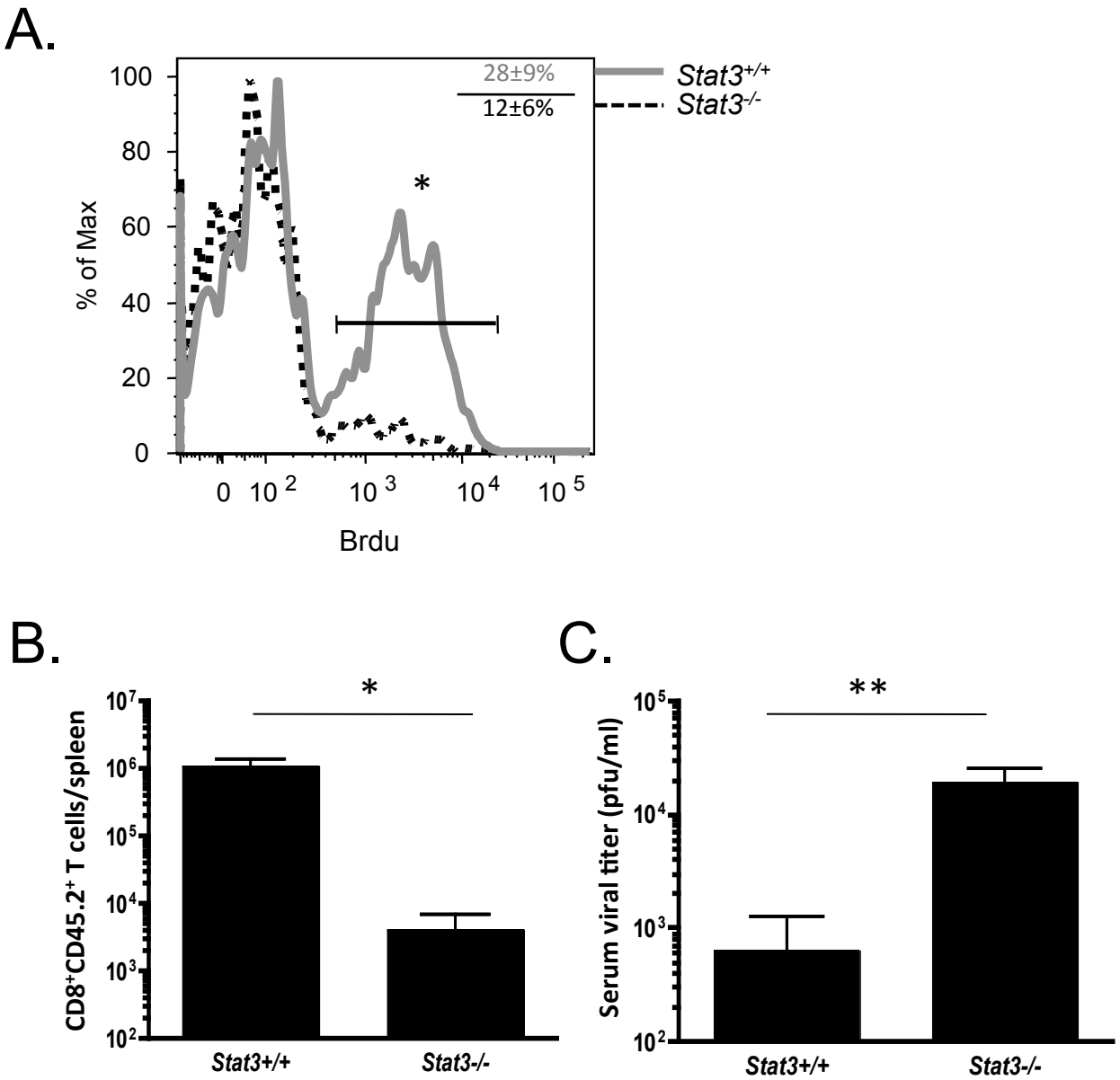
minutes. (B-D) WT and *Il21*$^{-/-}$ mice were infected with LCMV and either treated with αIL-10 mAb or mock injected with PBS for 8 days. (B) The numbers of total D^bGP_{33-41} tetramer$^+$ (open bars) and KLRG1^{lo} IL-7R^{hi} (black bars) CD8$^+$ T cells at day 8 p.i. are shown in the stacked bar graphs. (C) Dot plots show IFN-γ and TNF-α expression in day 8 effector CD8$^+$ T cells after stimulation with GP_{33-41} peptide for 5 hours prior to intracellular cytokine staining. (D) Bar graphs show viral titers in the serum at day 8 p.i. LOD denotes the level of detection. As a positive control for viral plaque formation, we also infected naïve mice with CL13 and their viral titers are shown as well. Data shown are representative of two independent experiments. (Statistical analyses show the comparison between KLRG1^{lo} IL-7R^{hi} groups, n.s. denotes "not significant").

Figure S4. The effects of IL-10 and IL-21 on Eomes, Tbet and BCL-6 expression in effector CD8 T cells. P14 effector CD8$^+$ T cells from day 6 LCMV infected mice were treated with IL-10 and IL-21 *in vitro* for 16 hours and the amount of Eomes, Tbet and BCL-6 expression (MFI) was measured by flow cytometry and plotted in bar graphs.

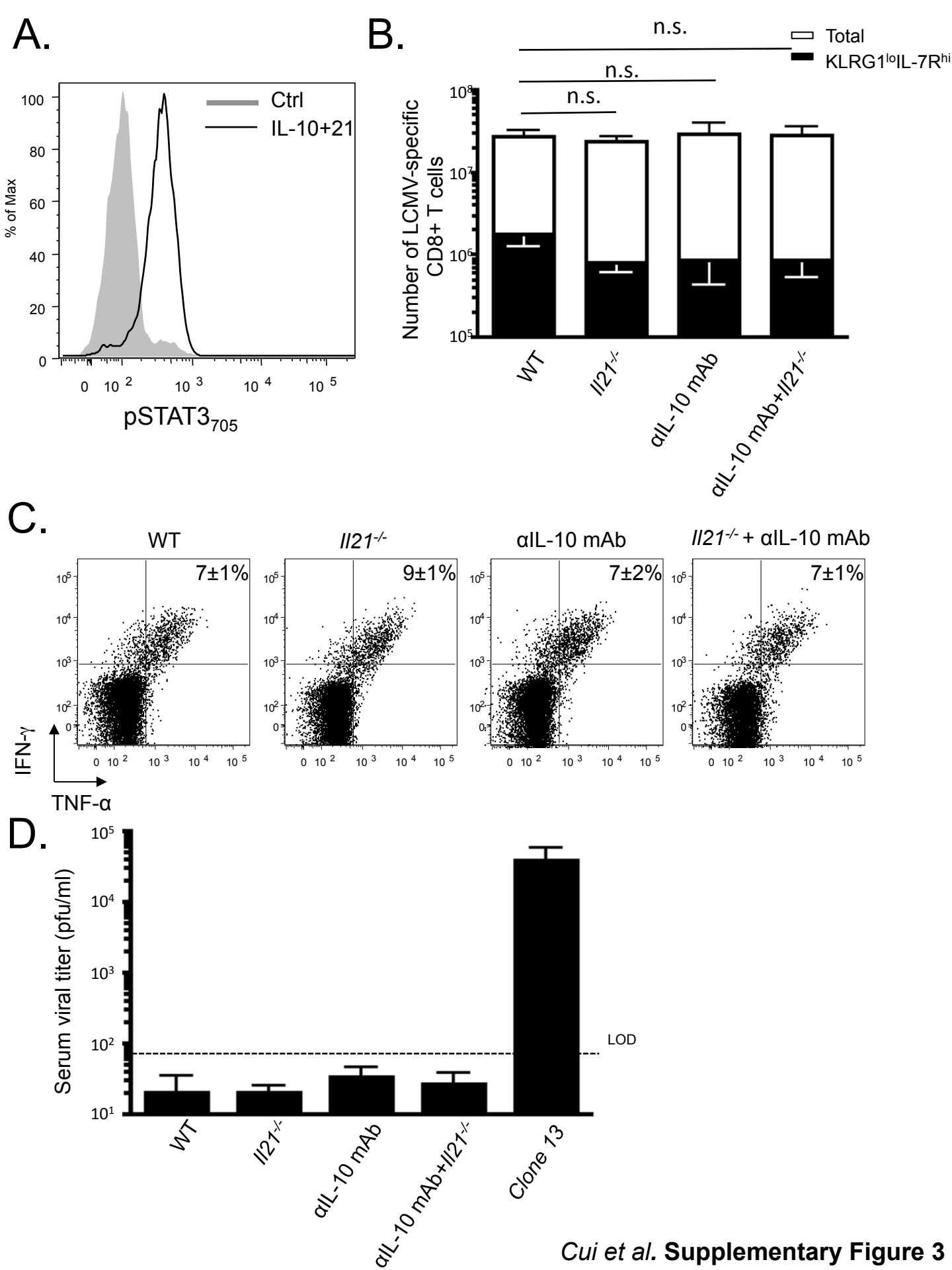
Figure S5. Elevated SOCS3 expression in effector CD8$^+$ T cells attenuates STAT4 activation. (A) Western blots show the amount of SOCS3 and Actin (loading control) expression in *Stat3*$^{+/+}$ or *Stat3*$^{-/-}$ P14 memory CD8$^+$ T cells isolated at day 40 p.i. by FACS sorting. Numbers below the graph indicate the normalized abundance of SOCS3 (from long exposure) measured by densitometry. (B) Plots show the amount of $pSTAT4_{693}$ in activated CD8$^+$ T cells that were either treated with IL-10 and IL-21 or left alone for 48 hrs and then stimulated with IL-12 for 30 minutes. (C-F) P14 CD8$^+$ T cells were transduced with control RV (MigR1) or SOCS3 shRNAi RV and adoptively transferred into C57BL/6 mice that were subsequently infected with LCMV. Eight days later the expression of SOCS3 (C) KLRG1 and IL-7R (D), Granzyme B (E), IFNγ$^+$ and TNFα$^+$ (F) was examined by flow cytometry. For (F), CD8 effector CD8 T cells were restimulated with GP_{33-41} peptide for 5 hours prior to intracellular cytokine staining. Data shown are representative of two independent experiments.



Cui et al. **Supplementary Figure 1**



Cui et al. **Supplementary Figure 2**



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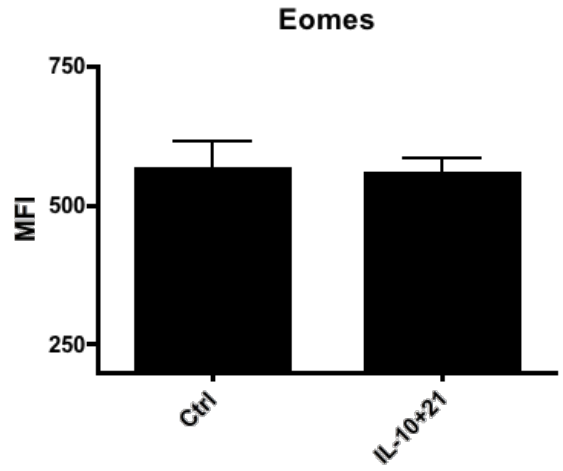
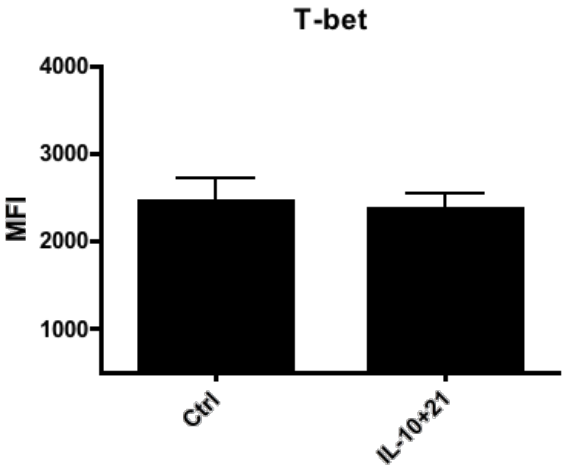
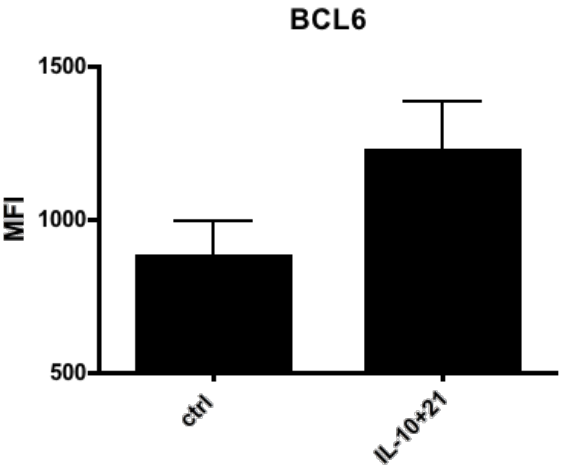
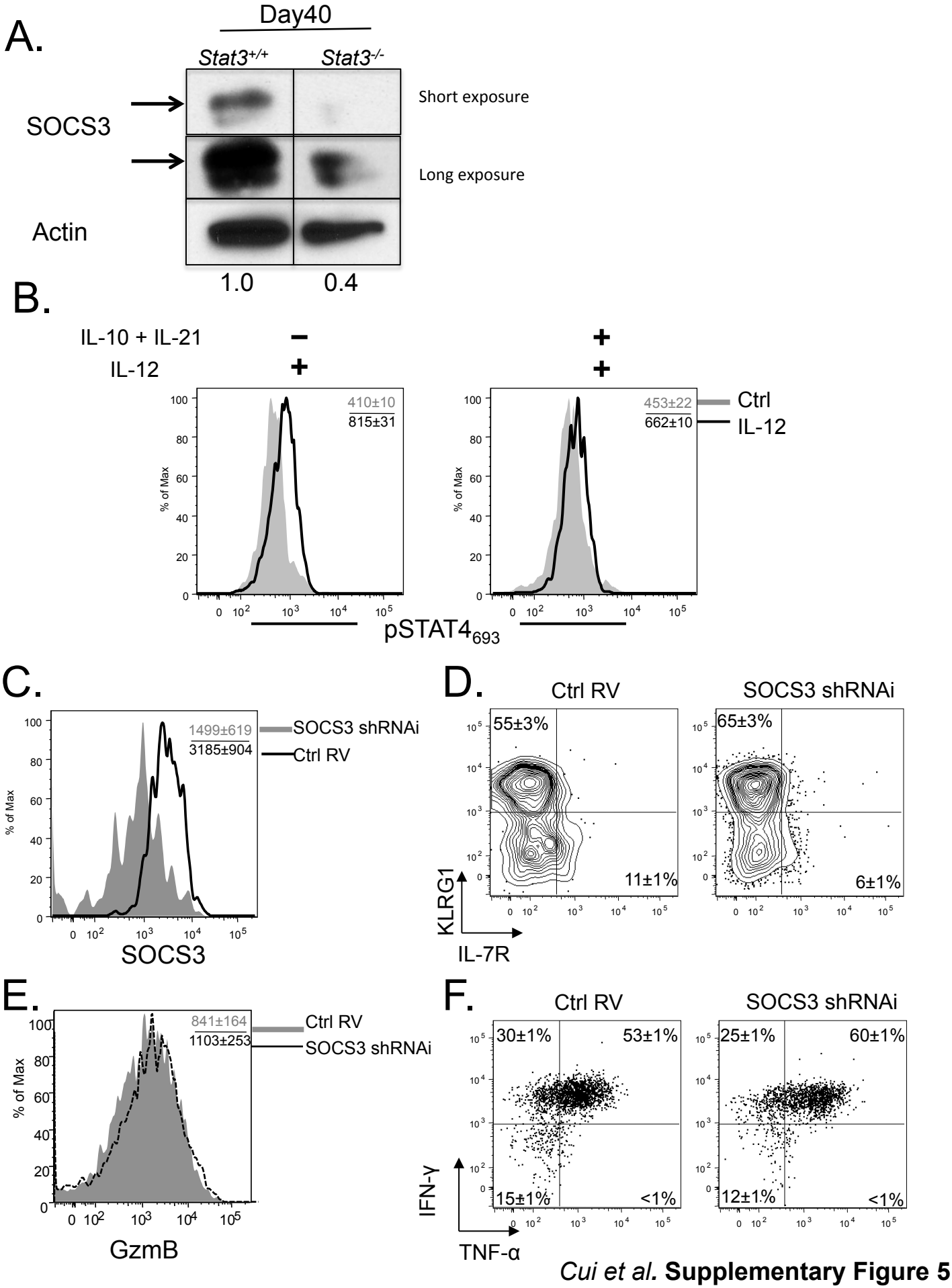






Cui et al. **Supplementary Figure 4**



Cui et al. **Supplementary Figure 5**